Nota Lepi. **45** 2022: 191–205 | DOI 10.3897/nl.45.80106 Research Article

Biology and DNA barcode analysis of *Coleophora lessinica* Baldizzone, 1980 and *Coleophora impalella* Toll, 1961 (Lepidoptera, Coleophoridae) with description of their larval cases

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http://zoobank.org/6ABA523B-081D-44D8-9466-A909DBB243D6

Received 4 January 2022; accepted 31 March 2022; published: 21 April 2022
Subject Editor: Lauri Kaila.

Abstract. Host plants and cases of several species in the genus *Coleophora* Hübner, 1822 have remained unknown until now, while the latter structures provide important characters for identification. Host plants and cases of *Coleophora lessinica* Baldizzone, 1980 and *Coleophora impalella* Toll, 1961 were discovered by the authors and are presented here for the first time. New data on the distribution and biology of the two species in Hungary are given. *Coleophora lessinica* is recorded as new for the fauna of Romania. DNA barcode sequencing was performed for both *Coleophora* species and loaded into the BOLD System and to GenBank.

Introduction

Members of the family Coleophoridae are distributed worldwide but the number of described species is highest in the Palaearctic region. The family currently includes more than 1500 described species in total (Baldizzone et al. 2006; Baldizzone and van der Wolf 2011, 2015, 2020a, b; Baldizzone 2019; Baldizzone 2021).

Based on the latest phylogenetic research (Bauer et al. 2012), only three genera are generally accepted. In Europe, three species are known in the genus *Augasma* Herrich-Schäffer, 1853, four in *Ischnophanes* Meyrick, 1891 and 621 in *Coleophora* Hübner, 1822 (Rennwald and Rodeland 2021). In Hungary the family comprises 211 species: one in *Augasma* and 210 in *Coleophora* (Pastorális and Buschmann 2018; Tabell and Kosorín 2020; Szabóky and Takács 2021).

Knowledge of the biology of *Coleophora* species is well-known in northern Europe but rather limited in southern Europe. The spectrum of host plants is wide and includes Asteraceae, Betulaceae,

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Caryophyllaceae, Cistaceae, Chenopodiaceae, Ericaceae, Juncacae, Fabaceae Lamiaceae, Poaceae, Rhamnaceae, Rosaceae, but the host plants of many species are still unknown (Baldizzone 2019).

Coleophora lessinica Baldizzone, 1980 is a southern European species described on the basis of specimens collected in the Lessini mountain near Verona (Baldizzone 1980). It is known from Bulgaria (Richter 2017), Croatia, France, Italy, and North Macedonia (Baldizzone 2019). The first Hungarian specimen was collected in Fejér county, Bucka-hegy near Csákberény (26.viii.2000) (Szabóky et al. 2009). Biology of this species is unknown, according to Baldizzone (2019) and Buschmann et al. (2014). The species is univoltine, flying from August to September in xerothermic habitats.

Coleophora impalella Toll, 1961 was described from Southern Russia (Toll 1961) based on a single old male specimen. Later the female was described from the mid-section of the river Volga by Anikin (2004). In addition, there is a population in the Karadag Nature Reserve in Crimea (Budashkin and Puzanov 2017) Outside the Volga region and Crimean Peninsula it has been found only in Bélmegyer, Hungary, from where both sexes were re-described (Baldizzone and Tokár 2008). The adults are diurnal and not attracted to artificial light (Szabóky et al. 2009). Other aspects of the species' biology are unknown.

DNA based identification is often useful, not just for species identification by non-specialists, but also to reveal association between different life stages and sexes of the same species which otherwise might be a very time demanding task. Here we give some details about the biology of two *Coleophora* species and describe their larval cases.

Materials and methods

Field work, record and rearing

All field work was undertaken in Hungary and in Romania near the Hungarian border (Map 1).

Larvae of the studied species, including overwintering stages, were reared on their host plants planted in the gardens of the authors. Escape of larvae was prevented with a tulle bag attached around the host plant. Shortly before the expected time of emergence each case was put in a separate plastic vial to maintain appropriate humidity.

Specimens are deposited in the private collections of G. Baldizzone, Cs. Szabóky and A. Takács. Three specimens of *Coleophora impalella* Toll, 1961 are to be deposited in the Hungarian Natural History Museum after the publication of this paper.

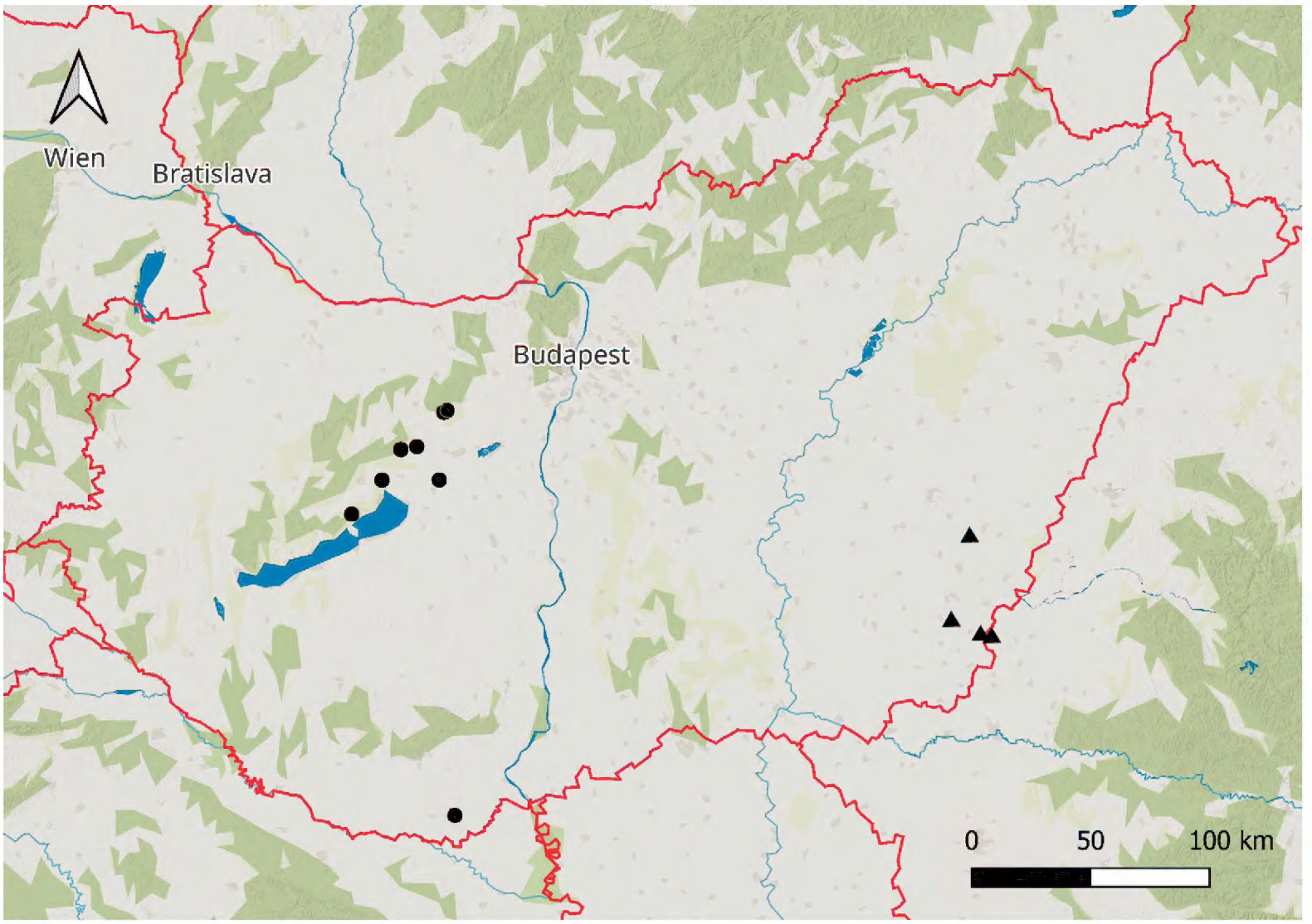
Acronyms

Bldz: genitalia preparation of Giorgio Baldizzone (Asti, Italy)
HNHM: Hungarian Natural History Museum, Budapest
IgR: genitalia preparation of Ignác Richter (Malá Čausa, Slovakia)
TB: genitalia preparation of Balázs Tóth (HNHM)

Morphological examinations

Photography and genitalia preparation

Images of intact larvae, adults and larval damage were prepared with a Canon 450 D camera, applied to a Carl Zeiss Stemi-2000 binocular stereomicroscope; they were edited with software Adobe Photosop CS6. The habitat image was taken with a Sony A7R2 camera.



Map 1. Collection localities of *Coleophora* specimens used in this study. Black dots: *C. lessinica*, black triangles: *C. impalella*. Map by Péter Gábor Sulyán (Budapest).

Methods for preparation of the genitalia follow van Nieukerken et al. (2012, 2018).
Images were adjusted with the software Adobe Photoshop CS6.

Molecular methods analysis

Four larvae of the two species were selected for molecular analyses (Table 1). Specimens were killed and stored in 70% alcohol. The genomic DNA was isolated from a piece of the larval body with Quick-DNA Tissue/Insect Miniprep Kit (Zymo Research) according to the recommended protocol of the manufacturer.

Amplification of the standard COI barcode region was performed with the primers LCO-1490 and HCO-2198 (Folmer et al. 1994). The PCR mixture was prepared and optimised according to the recommended protocol of the manufacturer (HotStarTaq Plus PCR Handbook, Qiagen). The PCR mixture contained 2µl of template DNA, 7.5µl of 2× HotStart Taq Plus Master Mix (HotStart Taq Plus Master Mix Kit, Qiagen), 0.5µl of each primer (10µM) and double distilled water to a final volume of 15µl. The amplification profile was determined based on the European and Mediterranean Plant Protection Organization PM 7/129 (2) protocol (Anonymous 2021) and modified for the use of the HotStart Taq Plus Master Mix Kit as follows: the profile consisted of a preheating step of 5 min at 95 °C followed by 5 cycles of 95 °C for 30 sec, 45 °C for 30 sec and 72 °C for 1 min, and additional 35 cycles at 95 °C for 30 sec, 51 °C for 1 min and 72 °C for 1 min

Table 1. *Coleophora* specimens from Hungary used for molecular analyses.

Species	Host plant	Locality	GPS coordinates	Date of collection	Collector	Stage	GenBank Accession number
C. lessinica	*Artemisia alba*	Csákberény	47°20'49"N, 18°21'19"E	12.x.2018	Attila Takács	Larva	MZ662875
C. lessinica	*Artemisia alba*	Gánt-Gránás	47°21'11"N, 18°22'17"E	09.x.2018	Attila Takács	Larva	MZ664319
C. lessinica	*Artemisia alba*	Nagyharsány	45°51'24"N, 18°24'52"E	04.xi.2018	Attila Takács	Larva	MZ662874
C. impalella	*Galatella sedifolia*	Szabadkígyós, Róka kaszinó	46°34'54"N, 21°05'00"E	17.viii.2021	Attila Takács	Larva	OK329945

and a final extension at 72 °C for 10 min. Reaction was performed in a PTC-100 DNA thermal cycler (MJ Research). Products of amplification were analysed in 2% agarose gel with addition of ethidium bromide and visualised under UV light. The PCR products were purified using the USB ExoSAP-IT PCR Product Clean-Up reagent (Affymetrix) and amplicons were sequenced bidirectionally (BaseClear B.V., The Netherlands). The sequences were assembled with Staden Package 2.0.0b9 (Staden et al. 1998). Sequences were inspected and translated in ExPASy Bioinformatics Resource Portal (Artimo et al. 2012) to verify that they are free of stop codons and that they are not pseudogenes.

To identify the larvae, two independent approaches were used. First nblast search was carried out in GenBank (www.ncbi.nlm.nih.gov/Genbank) optimized for highly similar sequences (megablast) (Accessed on 11/12/2021). Also, the identification engine of the BOLD homepage (www.barcodinglife.org) was used to search in the species level barcode library (Accessed on 11/12/2021). The graphical results obtained were visualised and edited with FigTree 1.4.4 (Rambaut 2018) and the final versions were created by Inkscape version 0.92 (Inkscape Project 2021).

Results and discussion

Rearing young larvae is difficult and cumbersome but has the advantage of limiting parasitism compared with starting with more advanced larvae. However, young larvae are very sensitive to the quality and quantity of food as well as to disturbance. Larvae in closed containers without ventilation are easily killed by mould, whereas in an open container the food plant easily becomes too dry to be palatable.

Perhaps the most sensitive period is when the larva changes its case. Natural conditions should be simulated as much as possible to enhance rearing success. The best approximation is to grow the host plants in a garden setting, place cases on them, and cover them with tulle bags to prevent larvae from escaping and to protect them from parasitoids. Success is not assured even when larvae reach the final L5 stage at which point they need to make a U-turn inside the case in preparation for pupation; many larvae fail to perform this turn and die. After overwintering, the larval stage lasts for an additional 4–5 months, with pupation taking place only 3–4 weeks before emergence of the adult. Despite of these difficulties, rearing is still the most efficient way to study the biology of these species.

Material examined

Coleophora lessinica Baldizzone, 1980

Figs 1–5, 7, 10–14

Adults. 1 ♀, **Hungary:** Fejér county, Kőszárhegy, 16.viii.2020, light trap. 1 ♀, same locality, 17.viii.2020, light trap; det. Giorgio Baldizzone (Asti), slide No. Bldz 17234 (Figs 10, 11). 1 ♀, Veszprém county, Litér, Mogyorós-hegy, 21.viii.2017, leg. Cs. Szabóky.

Cases. Hungary: 11 cases, Fejér county, Gánt-Gránás 09.x.2018, on *Artemisia alba* Turra, leg. A. Takács. 23 cases, same locality but 15.x.2019, on *Artemisia alba*, leg. A. Takács. 15 cases, same locality but 22.x.2020, on *A. alba*, leg. A. Takács. 25 cases, Fejér county, Csákberény, Bucka-hegy 10.x.2018, on *A. alba*, leg. A. Takács. 45 cases, same locality but 16.x.2019, on *A. alba*. leg. A. Takács. 10 cases, same locality but 22.x.2020, on *A. alba*, leg. A. Takács. 5 cases, Csákvár, Haraszt-hegy, 15.x.2021, on *A. alba*, leg. A Takács. 35 cases, Baranya county, Nagyharsány, Szársomlyó, 11.xi.2018, on *A. alba* subsp. *canescens*, leg. Cs. Szabóky, B. Tóth. 5 cases, same locality but 05.x.2019, on *A. alba* subsp. *canescens*, leg. Cs. Szabóky, B. Tóth. 3 cases, same locality but 22.x.2020, on *A. alba* subsp. *canescens*, leg. Cs. Szabóky, B. Tóth. 3 cases, Veszprém county, Balatonfüred, Nagymező, 10.x.2020, on *A. alba*, leg. Cs. Szabóky. 12 cases, Várpalota, Barbély-völgy, 18.x.2018, on *A. alba*, leg. Cs. Szabóky.

Coleophora impalella Toll, 1961

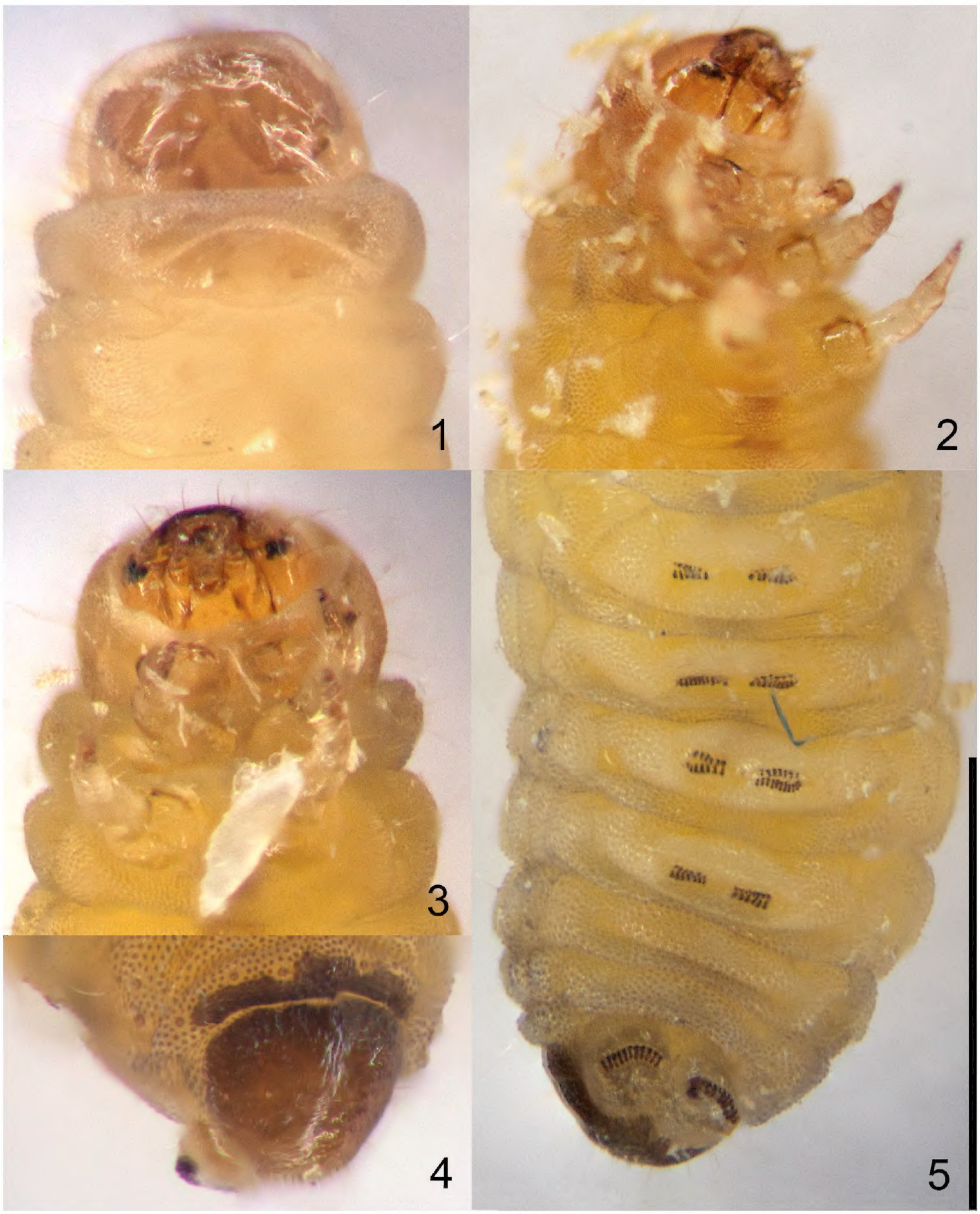
Figs 6, 8, 9, 18–26

Adults. 1 ♂, **Hungary:** Békés county, Bélmegyer, Fás puszta 29.iii.2021, ex larva, on *Galatella sedifolia* (L.), leg. A. Takács. 1 ♀, same data but 31.iii.2021. 1 ♀, same data but 13.iv.2021. 1 ♂, same data but 03.v.2021. 3 ♂ and 5 ♀, same locality but 12.v.2021, leg. Cs. Szabóky and A. Takács. 2 ♂ and 8 ♀, Békés county, Szabadkígyós, Kígyósi puszta, 12.v.2021, leg. Cs. Szabóky and A. Takács, slide Nos IgR31860 (Fig. 6), TB2148f, TB2149f (Figs 8, 9).

Cases. Hungary: 10 cases, Bélmegyer, Fáspuszta 24.ix.2020, on *G. sedifolia*. 3 cases, Békés county, Szabadkígyós, Kígyósi puszta, 17.viii.2021, on *G. sedifolia*, leg. A. Takács. 1 case, Békés county, Elek, 06.x.2021, on *G. sedifolia*, leg. G. Farkas. **Romania:** 1 case, Arad county, Grăniceri, 06.x.2021, on *G. sedifolia*, leg. G. Farkas.

Coleophora lessinica Baldizzone, 1980

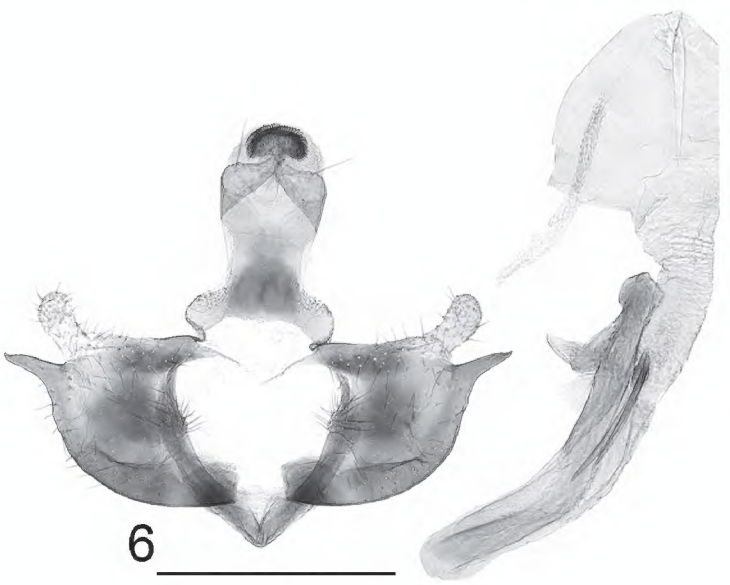
Larval morphology and recorded host plants. ***Case and larval behaviour.*** Initial case is made of a dry inflorescence of *A. alba*. The larva consumes the centre of the inflorescence, remaining parts are strengthened with silk (Fig. 12) and thus the remains of the inflorescence retain their original shape. Depending on its size, up to three or four larvae can feed on the same individual plant. Several inflorescences are consumed in the protection of this case, then the larva in L4 stage prepares a tubular silk case beneath the older one. Later on, the achaenia fall off from the tubular case (Figs 13, 14), and the larva seeks a still intact inflorescence, attaches the case to it, chews itself



Figures 1–5. Larval morphology of *Coleophora lessinica*. **1.** Head and thorax, dorsal view; **2.** Head and thorax, lateral view; **3.** Head and thorax, ventral view; **4.** Prolegs, ventral view; **5.** Anal plate, dorso-lateral view. Scale bar: 1 mm, cranial direction top, caudal direction bottom, photos by A. Takács.

inside the inflorescence, and performs its last moult in this shelter. Finally, it descends to the base of the host plant and attaches its case to it.

The mouth of case is at a right angle to the longitudinal axis of the case. Anal end of case is trivalved. Colour of case is light brown before overwintering, becoming dark brown after winter.



Figures 6–11. Genitalia of *C. impalella* and *C. lessinica*. **6.** Male genitalia of *C. impalella*, slide No. IgR 31860 (phallotheca right); **7.** Male genitalia of *C. lessinica*, slide No. IgR 23392 (phallotheca right); **8.** Female genitalia of *C. impalella*, signum broken, flattened, slide No. TB2148f; **8a.** Intact signum of *C. impalella* (enlarged), slide No. TB2149f; **9.** Abdominal segments of *C. impalella*, slide No. TB2149f; **10.** Female genitalia of *C. lessinica*, slide No. Bldz 17234; **11.** Abdominal segments of *C. lessinica*. Scale bars: 0.5 mm. Figs 6, 7: photo by Ignác Richter, figs 10, 11: photo by János Babics, figs 8, 9: photo by Balázs Tóth.

Overwintering happens at the base of the host plant. The larva climbs on the stem up to the basal-most leaves during August and September. There it attaches its case permanently to the stem, and this is the place where the larva turns around inside the case.

The early case, as well as the overwintering one, is very similar to that of *Coleophora absinthii* Wocke, 1877 (Figs 15–17), but the early case of *C. lessinica* is constructed from only

Figures 12–17. Cases of *C. lessinica* and *C. absinthii*. **12.** Flower case of *C lessinica*. Csákberény, Bucka-hegy, 20.x.2020, on *Artemisia alba*; **13.** Sheath case before overwintering of *C. lessinica*. Csákberény, Bucka-hegy, 27.x.2020, on *A. alba*; **14.** Sheath case before overwintering of *C. lessinica*. Csákberény, Bucka-hegy, 20.x.2020, on *A. alba*; **15.** Flower case of *C. absinthii*. Pákozd, 25.viii.2021, on *A. absinthium*. The larva is feeding in the third inflorescence, which will be incorporated to its case; **16.** Flower case of *C. absinthii*. Pettend, 30.viii.2021, on *A. absinthium*; **17.** Sheath case before overwintering of *C. absinthii*. Pettend, 04.ix.2021, on *A. absinthium*. Scale bars: 0.5 mm, photos by Attila Takács.

one inflorescence while that of *C. absinthii* is made of three flower heads that are unified before preparing the final, overwintering case.

L5 larva (Figs 1–5). Length 3 mm. Head brown. Body light brown. Thoracic shields (Fig. 1) and spiracular sclerites (Fig. 2) not sclerotised. Thoracic legs uniform light brown as body (Fig. 3). Prolegs

on segments A3–A6 with 12–18 crochets in two uniordinal rows; distribution of crochets asymmetrical (Fig. 5). Anal plate shiny brown (Fig. 4). Anal proleg half-moon-shaped, each with 11 crochets.

Habitat. The habitats of *C. lessinica* differ from each other in terms of bedrock. The bedrock in the habitats of Eastern Bakony Mountains (Kőszárhegy, Várpalota), the Balaton Uplands (Litér, Balatonfüred) and the Villány Mountains (Nagyharsány) is limestone, that of the Vértes Mountains (Csákvár, Csákberény, Gánt-Gránás) is dolomite. The bedrock affects the species of-plant associations, but the association-forming species everywhere in these habitats is *A. alba* (Borhidi 2003).

Coleophora impalella Toll, 1961

Case and behaviour. Construction of the case begins in late August or early September from the leaves of the host plant, *Galatella sedifolia*. This finding does not support Richter (2021), who has found cases on *G. linosyris* (L.).

The case is made of 2–6 mm long and 2–4 mm wide strips spun to each other with overlap, resulting in a sheath case (Figs 24, 25). Feeding lasts until drying of host plant in autumn. The overwintering case is 12 mm long and is attached to a broader section of the dried plant. The larva continues feeding on fresh leaves in spring. Early case is greyish black, changing to dark brown after overwintering. Mouth directed in 40 ° angle to longitudinal axis of case, case slightly tapering towards anal end, caudal 2/3 part slightly curved, anal end flattened, bivalved.

Adults emerge 10–12 days after the permanent attachment of the case. They fly in daytime and remain close to the host plant. Mating was observed between 11 h and 13 h.

L5 larva (Figs 18–23). Length 5.5 mm. Head brown, adfrontal suture black. Body light brown, with inconspicuous pinacula around prolegs on segments A3–A5. Thoracic shields shiny black, rounded; prothoracic shield ovoid, more than 1.5 times as broad as long, very finely divided along median axis except anterior margin; mesothoracic shield D-shaped, divided to two halves by gap medially, gap being narrowest at middle, shield gradually broadening towards anterior and posterior margins, anterior margin straight, lighter than rest of the shield; metathoracic shield made of two separated ovoid plates with dentate inner edges, plates separated by gap slightly broader than the plates (Fig. 18). Spiracular sclerites oval, of equal length, sclerite on prothorax half as broad as those on meso- and metathorax, latter two sclerites with uniform shape and size (Fig. 19). Thoracic legs brown; proximal parts of segments darker than distal parts (Fig. 20). Prolegs on segments A3–A5 with 6–6 crochets in two uniordinal rows; on segment A5 two crochets in anterior, four crochets in posterior row, three crochets in each of remaining row (Fig. 21). Anal plate matt black, heavily irrorated with tiny hollows (Fig. 23). Anal proleg half-moon-shaped, each with 17–19 crochets (Fig. 22).

Conditions of collecting. Ten cases of *C. impalella* were collected in Bélmegyer, Fáspuszta (Figs 24, 25), on 24.ix.2020. Dates and numbers of emerged specimens: 1 ♂, 29.iii.2021, 1 ♀, 31.iii.2021, 1 ♀, 13.iv.2021, 1 ♂, 03.v.2021.

In addition to the Hungarian locality already known, the species was also found in Szabadkígyós, Kígyósi puszta on 12.v.2021 (Fig. 26). One case was collected in Elek on 06.x.2021 and one case in Grăniceri, Romania on 06.x.2021. This latter record is the first one outside Russia and Hungary. New to Romania.

Habitat. *Coleophora impalella* feeds on *Asteretum sedifolii* Soó 1947 corr. Borhidi 1996 (Pannonic salt steppes and salt marshes), a species that is highly influenced by dry continental

Figures 18–23. Larval morphology of *Coleophora impalella*. **18.** Head and thorax, dorsal view; **19.** Head and thorax, lateral view; **20.** Head and thorax, ventral view; **21.** Prolegs on segments A3–A6, ventral view; **22.** Anal prolegs, ventral view; **23.** Anal plate, dorsal view. Scale bar: 1 mm, cranial direction top, caudal direction bottom, photos by A. Takács.

climate with extreme temperatures and uneven distribution of precipitation. This habitat type was formed by secondary salinisation (Fig. 27). It is characteristic for the Continental climate zone and can also be found in the Ukrainian and Russian steppe belt (Borhidi 2003).

Parasitoids. No cases of *C. impalella* and *C. lessinica* specimens have given parasitoid specimens in our rearings to date.

Molecular analysis and identification

The specimens from *Artemisia alba* all belong to the same haplotype. The alignment of the COI region numbered 536 nucleotide positions. The sequence length of the specimen from *Galatella sedifolia* was 595 nucleotides in the final data set.

Figures 24–27. Some stages and habitat of *C. impalella*. **24.** L5 case of *C. impalella* before overwintering, Bélmegyer, 13.x.2020; **25.** L4 case of *C. impalella* before overwintering, Bélmegyer 24.ix.2020; **26.** Imago of C. *impalella* from Szabadkígyós, Kígyósi puszta, 12.v.2021; **27.** Habitat of *C. impalella* in Szabadkígyós, Kígyósi puszta, 20.v.2021. Scale bar: 3 mm (**24**); 2 mm (**25**); 2.1 mm (**26**). Figs 24, 26: photo by A. Takács, Figs 25, 27: photo by G. Boldog.

No exact matches were found in GenBank (Table 2). In the specimen from *Galatella sedifolia* the closest hit with 99.5% similarity was a *C. impalella* specimen from Russia (KX048258). For all other hits, the similarity was below 95%. The specimen was identified by the BOLD System as *Coleophora impalella* (BOLD:ACB0657) with 99.5% probability (Fig. 28).

In case of the specimens from *Artemisia alba* the closest matches in GenBank were three specimens of *Coleophora artemisicolella* with 97.57% similarity (Table 3). The BOLD System based on similarity was not able to unambiguously identify our specimens from *Artemisia alba* because of the high similarity of related species (Table 4). The two possible matches were *C. lessinica* and *C. magyarica*. The tree-based identification tool indicated that our specimens belong to *C. lessinica* (BOLD:ABA2006) (Fig. 29). Molecular identification was confirmed by genitalia dissection (Figs 1–5).

The examination of DNA barcodes confirmed that *C. lessinica* and *C. impalella* are present with at least one population in Hungary. Based on our analysis, the barcodes of "*lessinica*" and "*impalella*" populations form monophyletic clusters. The studied DNA sequences of the Macedonian and Hungarian populations of *C. lessinica* were identical. The intraspecific distance between studied Russian and Hungarian *C. impalella* populations was 0.5% (3 nucleotide positions). Identification was confirmed by genitalia dissection (Figs 6–11).

We were able to collect *C. lessinica* specimens from every location where its host plant, *Artemisia alba* is known to occur in Hungary. This implies that the species is more widespread than the published records suggest. To clarify the distribution of the species the host plant populations

Table 2. First five hits in GenBank using MEGABlast search on the COI sequences of the Hungarian specimen identified as *Coleophora impalella*. Accessed on 11/12/2021.

Scientific Name	Max Score	Total Score	Query Cover	E value	Percent identity	Accession Length	Accession number
Coleophora impalella	1083	1083	100%	0.00	99.5	658	KX048258
Coleophora striolatella	911	911	100%	0.00	94.29	658	KX049535
Coleophora striolatella	900	900	100%	0.00	93.95	658	KX047871
Coleophora thurneri	889	889	100%	0.00	93.62	652	KX048570
Coleophora sp. JFL051	889	889	100%	0.00	93.62	658	HM406336

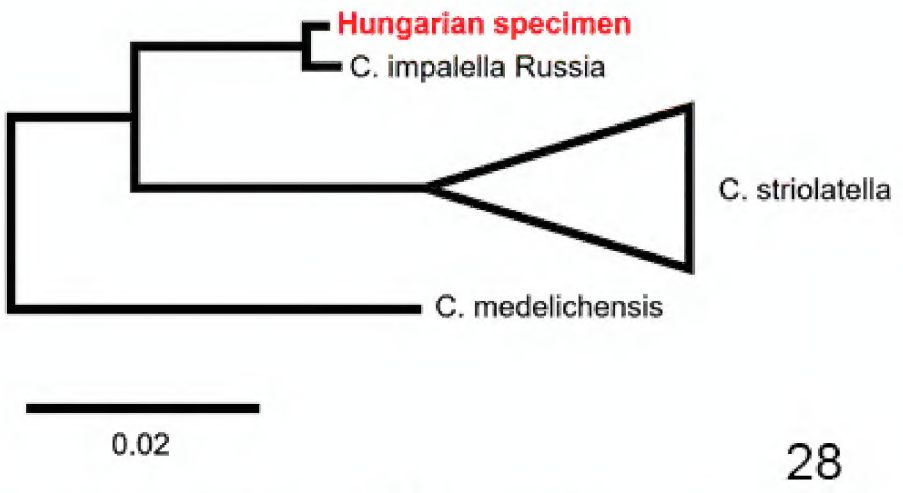


Figure 28. Part of the Neighbor-joining tree of the barcoded Hungarian specimen later identified as *Coleophora impalella*. Query sequence is marked with red. Scale bar indicates 2% K2P divergence of nucleotide substitution.

Table 3. First five hits in GenBank using MEGABlast search on the COI sequences of the Hungarian specimens later identified as *Coleophora lessinica*. Accessed on 11/12/2021.

Scientific Name	Max Score	Total Score	Query Cover	E value	Percent identity	Accession Length	Accession number
Coleophora artemisicolella	918	918	100%	0.00	97.57	672	JN248884
Coleophora artemisicolella	918	918	100%	0.00	97.57	658	HM871573
Coleophora artemisicolella	918	918	100%	0.00	97.57	658	HM875737
Coleophora fasciella	913	913	100%	0.00	97.39	658	MT394458
Coleophora nubivagella	907	907	100%	0.00	97.2	658	KX042273

Table 4. Top 20 matches from the BOLD System identification tool of the specimens later identified as *Coleophora lessinica*.

Genus	Species	Specimens	Similarity	Remarks
Coleophora	*lessinica*	2	100%	
Coleophora	*magyarica*	1	99.06%	private
Coleophora	*microdon*	1	97.94%	early-release
Coleophora	*artemisicolella*	8	97.57%	
Coleophora	*nubivagella*	8	97.57%	private

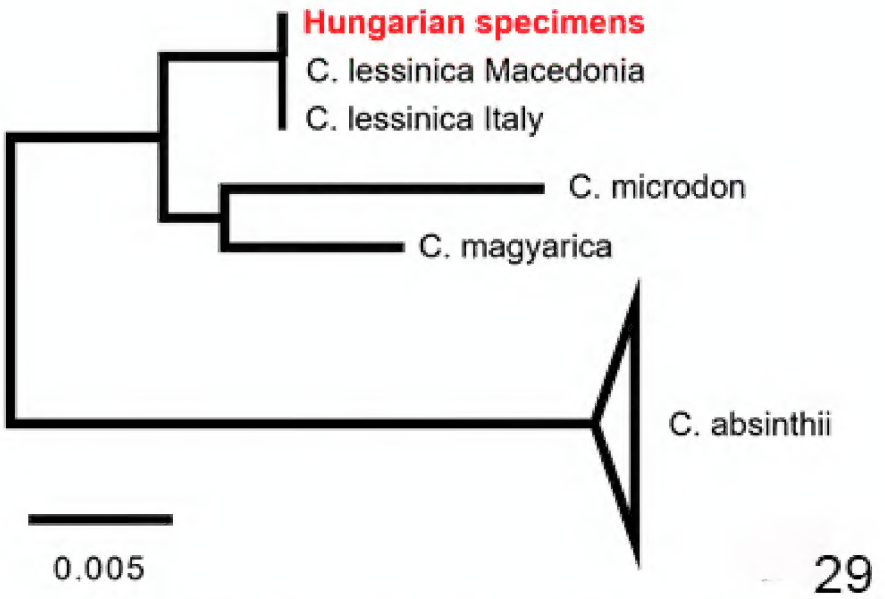


Figure 29. Part of the Neighbor-joining tree of the barcoded Hungarian specimen later identified as *Coleophora lessinica*. Query sequence is marked with red. Scale bar indicates 0.5% K2P divergence of nucleotide substitution.

should be investigated during the flowering period. A disjunct distribution of *C. impalella* is unlikely, due to the widespread occurrence of its host plant, *G. sedifolia*. With more comprehensive searches, it is likely that more populations will be discovered in the future.

Our results support that identifying material based on DNA barcodes is often highly reliable for this group and provides an alternative to morphology-based identification. DNA-based identifications are very useful and could be widely used in species identification when different life stages are difficult to connect. Nevertheless, morphological confirmations are still needed, especially when species have highly similar DNA barcodes.

Acknowledgements

We are grateful to those who helped us in searching for cases: Péter Finy, Diána Győri, István Harta and Kristóf Antal (Fejér County Government Office, Velence), Gábor Farkas (Békés County Government Office, Békéscsaba), Péter Gábor Sulyán (ÖMKI, Budapest). We thank Ignác Richter (Malá Čausa, Slovakia) for the genital preparations, and János Babics for genitalia photos. Special thanks to Giorgio Baldizzone (Asti, Italy) and Jean-François Landry (Ottawa, Canada) for their advice and professional help. We are indebted to Colin W. Plant (Bishops Stortford, UK) and the editor of Nota Lepidopterologica (David Lees) for linguistic corrections.

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